Balance, Excretion and Tissue Distribution of Vanadium in Rats after Short-Term Ingestion

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Balance, excretion and tissue distribution of vanadium in rats were investigated. Female Wistar strain rats, weighing 110–120 g (approximately 5 weeks old), were divided into a normal control group and a vanadium exposed group, of seven animals each. Vanadium exposed animals were fed the control diet plus sodium metavanadate (V^{5+}) at concentrations of 100 ppm for 7 days. Relatively high concentrations of vanadium were found in bone, spleen, kidney and liver in the vanadium exposed group, with the highest concentration found in bone. The percentages of vanadium excreted in the urine and feces were 0.86 and 83.5% of the intake, respectively. Thus, the retention rate was 15.7%. The data suggests that feces are the major route of excretion, and that vanadium is poorly absorbed by the intestinal tract.

Key words — vanadium, rat, retention, balance, bone

INTRODUCTION

Environmental pollution due to vanadium has recently become a serious problem.¹⁾ Vanadium in air originates mainly from the combustion of fuel oils, especially residual oils, which are known to be rich in this element, while the contribution of heavy vehicular traffic seems to be of minor importance according to experimental measurements and the knowledge of low vanadium levels in the distillate petroleum used in land transport. It has been shown that the burning of fossil fuels results in 2400–12000 tons

of vanadium per year, of which roughly 10–15% is deposited in the ocean as atmospheric fallout.²⁾ The toxicity of vanadium is largely confined to the respiratory tract. Irritant activity with respect to the skin and eyes has also been described from industrial exposure.³⁾ Although there is no direct information on toxicity levels for continuous year-round exposure, British sources report a statistical relationship between vanadium concentration in rural and urban air pollution and the incidence of lung cancer, bronchitis and pneumonia.⁴⁾ Epidemiological investigations have demonstrated a correlation between vanadium exposure and the incidence of lung cancer.⁵⁾ Athanassiadis⁶⁾ has reported at length on the toxicity of vanadium compounds and on the major sources of emissions. Several studies^{7–9)} have demonstrated that vanadium is a potent inhibitor of the ubiquitous enzyme Na, K-ATPase in erythrocytes and renal tubular cells of various species. Vanadium inhibits the biosynthesis of cholesterol¹⁰⁾ and phospholipid in livers of experimental animals. It is important to assess current and likely future intakes of vanadium *via* dietary, air and water sources. Furthermore, efforts to establish the point at which harmful effects begin to occur are necessary in the toxicological evaluation of vanadium. However, there is relatively little information on vanadium excretion, balance and tissue distribution following oral ingestion. Thus we examine these factors in this report.

MATERIAL AND METHODS

Animals and Diets — Female Wistar strain rats, weighing 110–120 g (approximately 5 weeks old), were purchased from Japan SLC Inc. (Shizuoka, Japan). After one week of acclimation to metabolic cages, the animals were fed *ad libitum* the control diet with or without vanadium as sodium metavanadate (100 ppm) for one week. This vanadium amount was set in consideration of the poor absorption of vanadium by the intestine. Sodium metavanadate was purchased from Wako Pure Chem. Ind., Ltd. Japan. Table 1 shows the composition of the control diet fed during the experiments. This diet was purchased from Oriental Co., Japan and values of nutrients were determined and reported by the same company. The vanadium content in the control diet was 0.32 ppm. After one week, the content of this

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Table 1. Composition of Control Diet

Component	Concentration (in 100 g of diet)	Component	Concentration (in 100 g of diet)
Protein	24.6 g	Vitamin B ₂	1.21mg
Fat	5.6 g	Vitamin B ₆	0.92mg
Carbohydrates	52.8 g	Niacin	9.68mg
Cellulose	3.1 g	Pantothenic acid	3.05mg
Calcium	1.15g	Folic acid	0.15mg
Phosphorus	0.88g	Choline	0.23g
Magnesium	0.25g	Inositol	433mg
Sodium	0.26g	Vitamin A	1810IU
Potassium	0.89g	Vitamin D ₃	80IU
Iron	12.3 mg	Vitamin E	10.4 mg
Vitamin B ₁	2.04mg		

All values were reported by Oriental Co.

element in various tissues was determined by a previously reported method.¹¹⁾ During feeding, feces and urine were collected daily.

Determination of Vanadium in Tissues — Tissue samples (1–0.1 g) were dried at 120°C for 2 h on a hot plate and ashed at 550°C for 6 h in a muffle furnace. The residue was dissolved in 5% HCl and pH adjusted to 4–6. The solution was transferred to a separatory funnel and 2 ml of each of 10% ascorbic acid and 1% ammonium pyrrolidine dithiocarbamate (PDCA) solution were added. After allowing the mixture to stand for 5 min, 5.0 ml of xylene was added and vigorously shaken for 2 min. The xylene layer was then subjected to an atomic absorption spectrophotometer with a graphic furnace atomizer.

Determination of Vanadium in Feces and Urine — All feces and urine collected were dried and reduced to ashes according to the method for tissues. After ashing, samples were dissolved in 5% HCl and diluted with distilled water. Since the vanadium concentrations were high, they could be analyzed directly by flameless atomic absorption spectrophotometry.

Statistical Analysis — Values are shown as means S.E.M., except where otherwise indicated. Data were analyzed using one-way ANOVA and, when appropriate, by a Student-Newman-Keul test. Results were considered significant at $p < 0.05$.

RESULTS

Recovery Tests

Our proposed method was applied to the determination of vanadium in various rat tissues. The recoveries of added vanadium in all samples were from 96.7 to 109.3%, with a maximum coefficient

Table 2. Recovery of Vanadium Added to Rats Tissues

Sample	Vanadium added ($\mu\text{g/g}$)	Recovery ^{a)} (%)	C. V. ^{b)} (%)
Liver	1.25	100.8 $\pm$ 1.4	1.4
Kidney	0.92	97.7 $\pm$ 1.1	1.1
Heart	2.22	109.3 $\pm$ 1.8	1.6
Lung	1.26	96.7 $\pm$ 2.3	2.4
Brain	1.25	99.0 $\pm$ 3.9	3.9
Thymus	5.88	100.2 $\pm$ 5.5	5.5
Spleen	2.85	103.3 $\pm$ 8.9	8.6
Muscle	1.33	99.1 $\pm$ 1.0	1.0
Intestine	1.50	98.8 $\pm$ 3.2	3.2
Stomach	1.26	100.3 $\pm$ 6.2	6.2
Femur	1.35	108.3 $\pm$ 0.5	0.5

^{a)} Values are expressed as means $\pm$ S.E. of five preparations.

^{b)} Coefficient of variation.

of variation of 8.6% ($n=6$) (Table 2).

Changes in Body Weights and Foods Intake

As shown in Fig. 1, there was no significant difference between the two groups in body weight gains throughout the feeding period. However, the vanadium exposed group showed a decrease in food intake on the fifth day of feeding. Table 3 shows vanadium intake, excretion and balance. The balance in the body was calculated by assuming that excretion by routes other than feces or urine was negligible. It is known that low-molecular-weight compounds are poorly excreted into bile, while compounds with molecular weights exceeding a minimum value (about 325) are excreted in appreciable quantities into bile.¹²⁾ From the molecular weight of vanadium, its excretion into the bile from liver was negligible. The daily intake of vanadium was estimated at 7060 $\mu\text{g/kg}$, and the percentages excreted in the urine and feces were 0.86 and 83.5% of the intake, respectively. The retention was 15.7%.

Table 3. Vanadium Excretion and Balance in Rats Fed Diets Containing Vanadium

	Vanadium ^{a)} ($\mu\text{g/kg/d}$)	(% of total intake)
Intake (A)	7060 $\pm$ 574	
Urinary excretion (B)	61.1 $\pm$ 13.9	(0.86%)
Fecal excretion (C)	5892 $\pm$ 845	(83.5%)
Retention (A-B-C)	1107 $\pm$ 520	(15.7%)
Absorption (A-C)	1168 $\pm$ 519	(16.5%)

^{a)} Values are expressed as means $\pm$ S.E. derived from seven rats. Vanadium levels are expressed as micrograms per kilogram body weight per day.

Fig. 1. Body Weights and Food Intake in Control Rats and in Rats Fed Diets Containing Vanadium

Data represent means $\pm$ S.E. of seven rats.

Food intake is expressed as grams per day per 100 g body weight.

*Significantly different from the control group ($p < 0.05$).

**Significantly different from the control group ($p < 0.01$).

Fig. 2. Vanadium Contents in Control Rats and in Rats Fed Diets Containing Vanadium

Results are expressed as means $\pm$ S.E. derived from seven rats.

Vanadium levels are expressed as micrograms per gram of tissue wet weight.

Vanadium Contents

Figure 2 shows the vanadium contents in control rats and in rats fed diets containing vanadium (100 ppm). Relatively high concentrations of the element were found in bone, spleen and kidney in the vanadium exposed group. The

highest was in the tibia ($1.84 \mu\text{g/g}$) and the lowest was in the brain ($0.13 \mu\text{g/g}$). Otherwise, the largest amount in the control group was found in the lung ($0.35 \mu\text{g/g}$).

DISCUSSION

A high percentage of ingested vanadium was recovered from feces in our study, showing that feces are the major route of excretion of orally administered vanadium while urine is the minor excretory pathway. The low percentage of ingested vanadium recovered from urine in our study is much lower than the mean of 5.2% found by Tipton *et al.*¹³⁾ in a study of 16 elderly humans. Talvitie¹⁴⁾ reported that the greatest elimination of vanadium occurred in urine within 24 h after injection, and that fecal elimination accounted for a much lower amount in rat. Studies of the excretion in subjects given vanadium orally showed that urine is the minor excretory pathway and feces are the major route of excretion.^{15,16)} It has generally been assumed that ingested vanadium is poorly absorbed from the gastrointestinal tract.¹⁷⁾ When intravenously injected into the bloodstream, excretion *via* urine is the major route. Our data shows considerable retention (16%) of this element by the body, and relatively high concentrations of it were found in bone. This is in direct opposition to the finding of Proescher and associates¹⁸⁾ who were unable to demonstrate the presence of vanadium in the bones of intravenously injected rabbits. Talvitie¹⁴⁾ reported that despite the rapid renal elimination of pentavalent vanadium, there was considerable retention of this element, at least for a few weeks; the element appears to be retained in the skeleton for the most part. In our study, vanadium content in the control rat was highest in the lung. This may be due to inhalation throughout the whole lifetime from birth. Byrne and Kosta¹⁹⁾ reported that in rats the lungs accumulated major portion of the increased intake, and the concentration in this organ increases with age. Browning³⁾ reported that U.S. city dwellers showed increased lung concentrations of vanadium in the fifth and particularly sixth decades of life and intake *via* air may be significant. Small amounts of vanadium can be found in some tissues in addition to the lung in the control rats. This can be attributed to the fact that the control diet contained 0.32 ppm vanadium. The findings of this study showed that relatively high concentrations of vanadium were found in bone in rats

fed a diet containing vanadium and therefore suggests that vanadium may affect the hemopoietic system. We confirmed this possibility by rat experiments. Details of these experiments will be described elsewhere.

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